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Nanoscale presentation of cell adhesive molecules via block copolymer self-assembly

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ABSTRACT

Precise control over the nanoscale presentation of adhesion molecules and other biological factors represents a new frontier for biomaterials science. Recently, the control of integrin spacing and cellular shape has been shown to affect fundamental biological processes, such as differentiation and apoptosis. Here, we present the self-assembly of maleimide functionalised polystyrene-block-poly (ethylene oxide) copolymers as a simple, yet highly precise method for controlling the position of cellular adhesion molecules. By manipulating the phase separation of the functional PS-PEO block copolymer used in this study, via a simple blending technique, we alter the nanoscale (on PEO domains of 8–14 nm in size) presentation of the adhesion peptide, GRGDS, decreasing lateral spacing from 62 nm to 44 nm and increasing the number density from ~450 to ~900 islands per μm^2. The results indicate that the spreading of NIH-3T3 fibroblasts increases as the spacing between domains of RGD binding peptides decreases. Further, the same functional PS-PEO surfaces have been utilised to immobilise, via a zinc chelating peptide sequence, poly-histidine tagged proteins and extracellular matrix (ECM) fragments. This method is seen as an ideal platform for investigations into the role of spatial arrangements of cell adhesion molecules and ECM molecules on cell function and, in particular, control of cell phenotype.

1. Introduction

For anchorage dependent cells, integrin mediated adhesion to extracellular matrix components is critical for many cellular functions, such as regulation of cell morphology, migration, growth, differentiation and apoptosis.

Controlling the shape of a cell, through controlled patterning of adhesive extracellular ligands, has been shown to control the cells ability to survive and proliferate [1]. Furthermore, cell shape has been shown to regulate the lineage specification of human mesenchymal stem cells [2]. These findings were facilitated by developments in biomaterials technology, namely micro-contact printing developed by Whitesides et al. [3], that allowed patterning of adhesive ligands on length scales from 5 μm and larger. However, micro-contact printing does not provide precise spatial control over individual integrin binding sites, and thus, no information regarding the role of integrin spacing can be obtained from this, or similar, techniques.

More recently, work from Spatz et al. has been focused on determining the role that the precise nanoscale location of integrin binding sites has on cell behaviour [4,5]. The motivation for this work is the recognition that the presentation of integrin binding sites *in-vivo* is controlled with exquisite precision on the nanometre length scale; for example, collagen fibres interact with a spatial periodicity of 67 nm with tissue cells *in-vivo* [6]. Spatz et al. utilised block copolymer micelle nanolithography to generate regular arrays of gold nanoscale dots that were then utilised as binding sites for thiol containing adhesion motifs. Their findings indicate that cell spreading and development of focal adhesions are affected by the nanoscale spacing of integrin adhesion sites. This highlights how improvements in biomaterials technologies facilitate the further understanding of fundamental biological processes. Further development of simple, versatile, but highly precise biomaterials technologies for the control of adhesive ligand presentation and extracellular matrix mimicry will thus ultimately allow for further elucidation and control of fundamental biological interactions.

Here we present the rapid self-assembly of asymmetric block copolymers of PS-PEO, where the terminal alcohol of the PEO block has been modified to carry a functional maleimide group, as a simple method to precisely control the nanoscale presentation of

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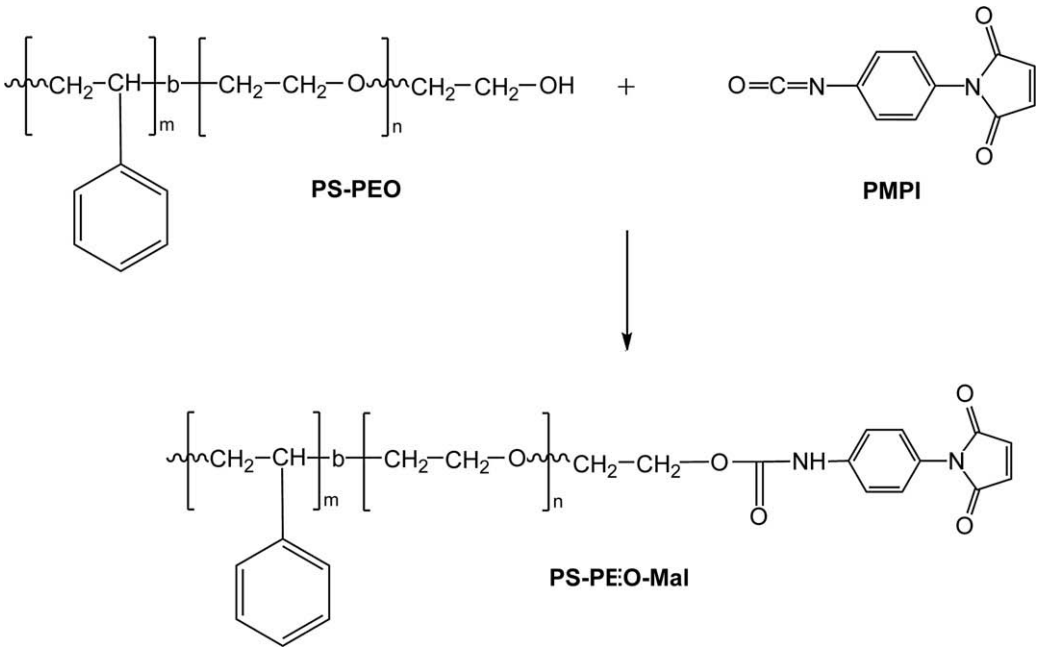

Fig. 1. Reaction of polystyrene-block-poly (ethylene oxide) (PS-PEO) copolymer with *N*-(p-Maleimidophenyl)isocyanate (PMPI) to generate a PS-PEO copolymer where the terminal alcohol group of the PEO block has been converted to a maleimide group.

cell adhesive ligands. The self-assembly of asymmetric PS-PEO block copolymers results in the formation of highly ordered cylindrical nanoscale domains of PEO dispersed in a background of PS, and has been extensively investigated by Russell et al. [7] as well as by our group [8,9]. Here, we extend on this previous work by introducing a functional terminal group to the PEO component, in this case a maleimide group that is capable of binding specifically to an amino-acid cysteine. Critically, we demonstrate that the micro-phase separation behaviour of the modified PS-PEO copolymer is not affected by the addition of this terminal functional

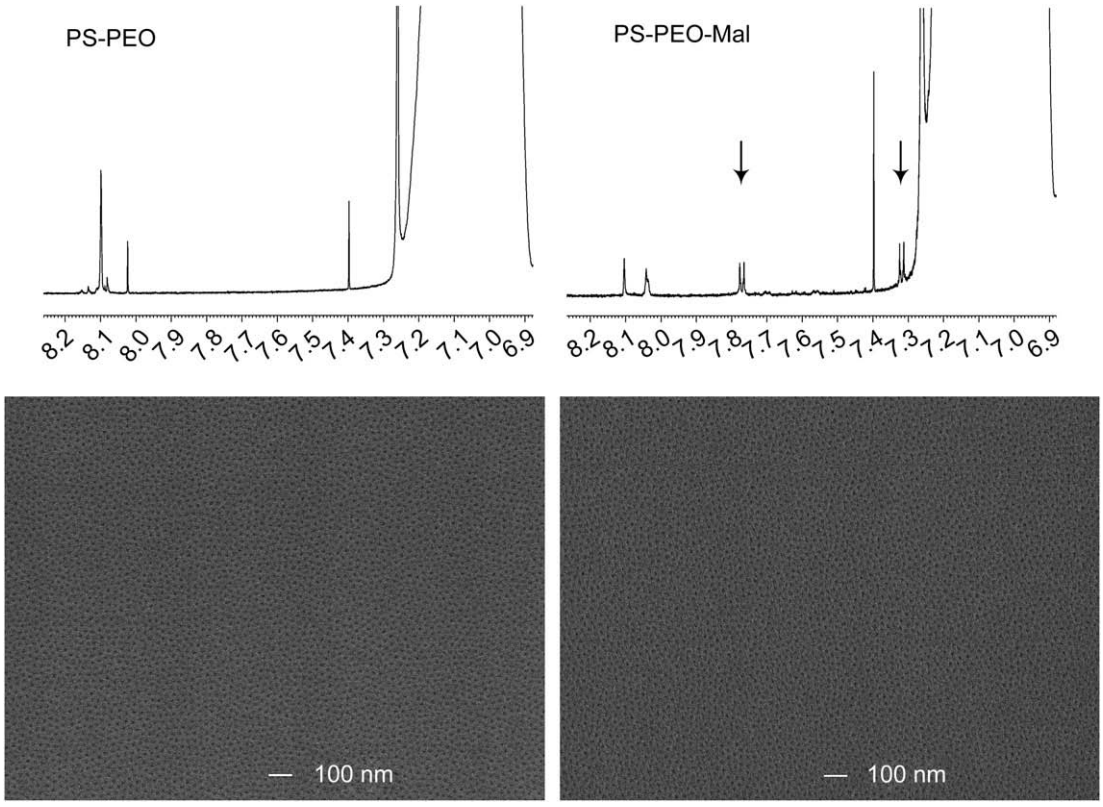

Fig. 2. High resolution ^{1}H NMR spectra for the non-modified PS-PEO polymer (left) and the modified PS-PEO-mal polymer (right). The doublet peaks at 7.76 and 7.32 are from the phenyl group of the PMPI after reaction with the PS-PEO. The lower images are SEM micrographs of the surface morphology of spin cast films from PS-PEO (left) and PS-PEO-mal (right) indicating that the addition of a maleimide end group does not affect the polymers phase-separation behaviour.